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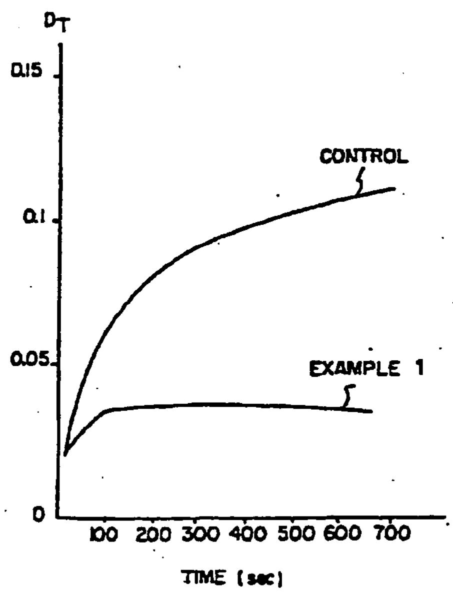
(54) **Analytical element containing photosensitive compound and filter layer and method of use.**

(57) A multilayer analytical element for the determination of a clinically significant enzyme analyte comprises a photosensitive compound (for example, a photosensitive dye or dye precursor) and a filter layer containing one or more filter dyes. The filter layer is situated in the element such that incident radiation used to detect a density change resulting from interaction of the analyte and the photosensitive compound passes through the filter layer before it reaches the photosensitive compound. The use of the filter layer inhibits premature changes in the photosensitive compound caused by incident radiation. This element is particularly useful for the determination of creatine kinase or one of its isoenzymes, for example, creatine kinase-MB.



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ANALYTICAL ELEMENT CONTAINING PHOTOSENSITIVE COMPOUND AND FILTER LAYER AND METHOD OF USE

The present invention relates to clinical chemistry. In particular, it relates to a multilayer analytical element useful for determination of clinically significant enzyme analytes, for example, lipase, creatine kinase or an isoenzyme thereof. This invention also relates to a method of using such analytical element.

Colorimetric assays of various fluids for the determination of chemical or biological substances (identified as analytes herein) are well known. Such assays are particularly important in clinical chemistry as the medical and veterinary professions attempt to rapidly and economically diagnose and treat ailments in humans and animals. As a result, researchers are continually searching for more sensitive and less expensive means for doing such assays.

A relatively recent contribution to the clinical chemistry art was the development of thin-film multilayer analytical elements. Those elements are generally described as having a porous spreading layer, a reagent layer and a registration layer carried on a nonporous support. The support may be designed to transmit all or part of incident radiation in order to facilitate measurement of detectable species at particular wavelengths.

Another significant advance in the art is the multilayer element described in U.S. Patent 4,089,747. This reference describes certain triarylimidiazole leuco dyes which have become very useful in assays for hydrogen peroxide or glucose, uric acid and other analytes where hydrogen peroxide is generated as a result of the presence of the analyte.

The determination of the activity of creatine kinase (abbreviated herein to CK, but also known as creatine phosphokinase, CPK, or ATP:creatine phosphotransferase E.C.2.7.3.2.) in human serum is considered one of the most sensitive laboratory methods for diagnosing diseases of skeletal muscles and diseases of the myocardium. CK determinations are useful, for example, for diagnosis of progressive muscular dystrophy, dermatomyositis and especially myocardial infarctions. Determination of CK-MB, one of the three isoenzymes of CK, is important for the evaluation of the damage to the heart in the case of cardiac infarctions.

Most standard assays for a number of analytes, including creatine kinase, generally measure a change in light absorption. Light incident on the test sample can be either broad band radiation or filtered radiation, depending upon the optical equipment and procedure used.

It has been discovered however, that some compounds used in such assays are photosensitive, that is, they change prematurely in response to light. In particular, some dyes or dye precursors useful in assays (for example, the triarylimidazole leuco dyes described in U.S. Patent 4,089,747) exhibit undesirable photosensitivity in various assays, including assays for CK or other enzymes. As a result, the dyes or their precursors prematurely provide an unwanted optical density change and a high rate of background formation in the assay. In other words, there is an unwanted detectable change in rate. This problem was not recognized in assays of analytes which are present in high concentrations because the response from the analyte is so much greater than the unwanted background. However, the problem became pronounced in instances where the analyte is present in relatively low concentrations.

A high rate of background formation significantly reduces assay sensitivity and precision.

While many photosensitive dyes or dye precursors, such as leuco dyes, are useful for assays of low level analytes, their use may be restricted due to their photosensitivity.

The problems noted above are overcome with a self-supporting analytical element comprising an absorbent carrier material containing an interactive composition for a clinically significant enzyme analyte comprising a photosensitive compound,

the element being characterized as having a filter layer comprising at least one filter dye and situated in relation to the carrier material such that incident radiation for detecting the density change passes through the filter layer prior to incidence upon the photosensitive compound.

In a preferred embodiment, a multilayer analytical element contains an interactive composition comprising a photosensitive dye or dye precursor which is capable of providing an optical density change as a result of interaction with a clinically significant enzyme analyte, the element comprising a support having thereon one or more layers, one of which contains the photosensitive dye or dye precursor,

the element being characterized as further comprising a filter layer comprising at least one filter dye and situated in relation to the layer containing the dye or dye precursor such that incident radiation for detecting the density change passes through the filter layer prior to incidence upon the layer containing the photosensitive dye or dye precursor.

More specifically, this invention provides a multilayer analytical element for the determination of total creatine kinase or an isoenzyme thereof containing an interactive composition comprising a photosensitive dye or dye precursor which provides a detectable optical density change upon interaction with creatine kinase, the element comprising a nonporous support having thereon:

a first layer containing the photosensitive dye or dye precursor, and

an outermost porous spreading layer,

the element being characterized as further comprising a filter layer comprising at least one filter dye and situated in relation to the first layer such that incident radiation for detecting the density change passes through the filter layer prior to incidence upon the first layer.

A method for the determination of a clinically significant enzyme analyte comprises the steps of:

A. contacting a sample of a liquid suspected of containing a clinically significant enzyme analyte with an analytical element as described above, and

B. determining the optical density change resulting from the presence of the analyte.

The present invention provides a highly sensitive spectrophotometric assay for a clinically significant enzyme analyte of choice in which a photosensitive compound (for example, a dye or precursor) is used. Clinically significant enzyme analyte is a term of art known to refer to those enzymes which are of interest in clinical evaluation of human or animal biological fluids. Such enzymes are normally in biological fluids in measurable amounts.

The present invention overcomes the problem of unwanted background rate which is particularly severe in the detection of low level enzyme analytes. Determinations of isoenzymes, for example, creatine kinase-MB, can be determined to advantage with this invention. The assay of the present invention provides improved precision, particularly in the determination of CK-MB.

The advantages of the present invention are attained by the use of a filter layer in the element of the invention. This layer contains one or more filter dyes which absorb unwanted electromagnetic radiation that would adversely affect photosensitive compounds in the element. Unwanted background rate of reaction is thereby reduced and precision is improved. Element keeping is also improved with use of the filter layer. The filter layer can be placed in any location in the element as long as it is situated in the path of incident radiation used to detect the optical density change resulting from analyte reaction. This incident radiation passes through the filter layer prior to reaching the photosensitive compound.

FIGS 1 and 2 are graphical plots of transmission density (D_T) versus time for a measurement of premature formation of background as described in Examples 1 and 2 below.

FIG 3 is a calibration curve of CK-MB concentration versus rate of reaction x 10^{-1} as described in Example 3 below.

The present invention relates to the determination (qualitative or quantitative measurement) of any of a wide number of clinically significant enzyme analytes which can be determined spectrophotometrically. Such analytes are generally present in test fluids at low levels, for example,

less than 150 I.U./dl, and more particularly at less than 50 I.U./dl. They include creatine kinase or an isoenzyme thereof, lipase, lactate dehydrogenase, aldolases, transaminases and others known to one skilled in the art. This invention is particularly useful for the colorimetric determination of total creatine kinase or a creatine kinase isoenzyme in aqueous liquids.

The invention can be used to assay any aqueous fluid. It is particularly useful for the assay of animal or human biological fluids. Such fluids include, but are not limited to, whole blood, plasma, sera, lymph, bile, urine, spinal fluid, sputum, perspiration and the like as well as stool secretions. It is also possible to assay fluid preparations of human or animal tissue such as skeletal muscle, heart, kidney, lungs, brains, bone marrow, skin and the like. The preferred use of this invention is to determine an analyte in human blood serum. The test sample can be diluted or undiluted.

In a preferred embodiment, the present invention relates to an immunochemical method for selectively determining an isoenzyme of creatine kinase, for example, creatine kinase-MB, in a biological fluid which also possibly contains CK-MM and CK-BB. The other isoenzymes can be similarly determined. Generally, the method of this invention comprises appropriately contacting the liquid to be assayed with the analytical element of this invention, the details of which are provided below. Prior to or simultaneously with that contact, for an assay of an isoenzyme, the liquid sample is contacted with one or more antibodies which are capable of either preferentially reacting with or preferentially inhibiting the enzymatic activity of the isoenzymes not of interest, for example, the M subunits in the

CK-MM and CK-MB isoenzymes present in the sample. In this example, the B subunit of the CK-MB isoenzyme is ideally uneffected by the presence of the antibodies, and therefore is free to react in any of a number of reaction schemes to produce a detectable optical density change. The amount of CK-BB is generally considered negligible in such assays. The density change produced is then directly correlated to the amount of CK-MB isoenzyme in the fluid sample.

The method of this invention is carried out by measuring the optical density change generated as a result of the presence of the analyte when it is contacted and mixed with the reagents sufficient to produce the density change. In some cases, only a photosensitive dye or dye precursor described herein is needed to provide the optical density change. In preferred embodiments, additional reagents are in the element in the form of an interactive composition which, through one or more reactions, provides the optical density change in the presence of an analyte.

Photosensitive compounds which can be used in the method of this invention without concern about undesired photosensitive effects include any organic or inorganic compound which changes in some manner in response to electromagnetic radiation. For example, the compound could be a photosensitive dye or dye precursor which changes in optical density with incident radiation. Alternatively, the compound could be an electron transfer agent, cofactor, substrate, buffer, activator or antioxidant which changes, reacts or causes change in response to electromagnetic radiation.

Photosensitive dyes or dye precursors useful in the practice of this invention include all organic compounds which have the capability of absorbing or

emitting a characteristic wavelength for detection, or which can be converted to such species. These materials are adversely sensitive to electromagnetic radiation, particularly that in the 200 to 700 nm region of the electromagnetic spectrum and exhibit an optical density change in response to that radiation.

Classes of useful photosensitive dyes and precursors include: cyanines, allopolarcyanines, triarylmethanes and imidazoles, particularly di- and triarylimidazole leuco dyes such as those described in U.S. Patent 4,089,747, noted above, E.P. Application 122,641 and Japanese Patent Publication 58-045,557. The triarylimidiazole leuco dyes of U.S. Patent 4,089,747 are preferred in the practice of this invention.

The remainder of the details of this invention will be illustrated as it applies to an assay for creatine kinase or an isoenzyme thereof. However, this invention is not limited in scope to these embodiments.

The density change for the determination of creatine kinase is detected spectrophotometrically, meaning as an optical density resulting from the reaction of creatine phosphate or its reaction product according to the reaction (1) in the forward direction:

(1) Creatine phosphate + ADP $\xrightleftharpoons{CK}$ creatine + ATP.

In its simplest form, the assay can measure either the disappearance of creatine phosphate, or the appearance of creatine.

Generally, however, reaction (1) is coupled with one or more other enzymatic reactions which provide an optical density change as a result of further reaction of ATP or its reaction product. The

optical density change can be colorimetric, fluorometric, or photometric, and can be either a change from colorless to colored, a change from colored to colorless, a change in the rate of increase or decrease in optical density, or a shift in absorbance from one wavelength to another.

More particularly, total CK or an isoenzyme, for example CK-MB, is determined by colorimetric means whereby an optical density change is measured at a wavelength between 200 and 900 nm.

In this embodiment, the optical density change is provided by a photosensitive dye or dye precursor which reacts with the byproducts of the reaction of the analyte with an interactive composition.

In a preferred embodiment of the present invention, total CK or CK-MB activity is determined by the following sequence of reactions:

(1) Creatine phosphate + ADP $\xrightarrow{\text{CK}}$ creatine + ATP

(2) ATP + glycerol $\xrightarrow{\text{glycerol kinase}}$ L-α-glycerophosphate + ADP

(3) L-α-glycerophosphate + electron acceptor $\xrightarrow{\text{α-glycerophosphate oxidase}}$ dihydroxyacetone phosphate + intermediate species (for example, H_2O_2)

(4) Intermediate species + photosensitive dye or dye precursor $\longrightarrow$ colorimetrically detectable species.

Quantification of total creatine kinase or its isoenzyme in the practice of this preferred embodiment is achieved using oxygen as the electron acceptor, a substance having peroxidative activity, and a photosensitive chromogen. In such a case, reaction (3) produces dihydroxyacetone phosphate and hydrogen peroxide. The details of this sequence of reactions are provided in U.S. Patent 4,547,461.

Useful peroxidative substances include peroxidase, both naturally occurring and synthetic. A peroxidase is an enzyme which will catalyze a reaction wherein hydrogen peroxide oxidizes another substance. The peroxidases are generally conjugated proteins containing iron porphyrin. Peroxidase occurs in horseradish, potatoes, fig tree sap and turnips (plant peroxidase), in milk (lacto peroxidase), and in white blood corpuscles (verdo peroxidase). It also occurs in microorganisms and can be produced by fermentation. A preferred peroxidase is that obtained from horseradish. Other peroxidative substances are known in the art.

Photosensitive chromogens which provide color formation in the presence of hydrogen peroxide and peroxidase useful in the present invention are described above. Leuco dyes are particularly useful including those described in the references noted above. Particularly useful leuco dyes include 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl)imidazole, 2-(4-hydroxy-3-methoxyphenyl)-4,5-bis(*p*-dimethylaminophenyl)-1H-imidazole, 2-(3-ethoxy-4-hydroxyphenyl-4,5-bis(*p*-dimethylaminophenyl)-1H-imidazole, 2-(4-hydroxy-3,5-dimethoxyphenyl)-4-[4-(dimethylamino)-phenyl]-5-(2-furyl)-imidazole, 2-(4-hydroxy-3,5-dimethoxyphenyl)-4,5-di(2-furyl)imidazole, 2-(3,5-dimethoxy-4-hydroxyphenyl)-4-[4-(dimethylamino)phenyl]-5-phenethylimidazole and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4-[4-(dimethylamino)phenyl]-5-benzylimidazole.

The amounts of the reagents, substrates and enzymes useful in the practice of this invention, including the photosensitive dyes or dye precursors, are dependent to a large extent upon the concentration of enzyme analyte (for example,

creatine kinase or isoenzyme) in the sample, the sophistication of the detection apparatus, and the detectable change produced. The amounts are readily determinable by one skilled in clinical chemistry having the teachings of the references noted above before him.

The analytical element of this invention can also include other reagents or addenda generally used in total CK or CK isoenzyme determinations, including CK activators, adenylate kinase inhibitors, metal ion cofactors (for example, magnesium, calcium and iron ions), solvents, buffers or surfactants. It is particularly desirable to include one or more CK activators which promote full creatine kinase activity. Such activators include mercapto-containing compounds (also known as thiol-containing or sulfhydryl compounds), such as thioglucose, dithiothreitol, dithioerythritol, mercaptoethanol, glutathione, N-acetylcysteine, cysteine, thioglycerol and thioglycolic acid in amounts known to one skilled in clinical chemistry.

Antibodies useful in the practice of this invention to determine a CK isoenzyme can be specific to either B or M subunits and can be generated from antisera using known procedures. The antibodies are generally used on a suitable carrier. In the assay of this invention, one or more antibodies can be immobilized within the element itself, if desired, without any additional carrier material or added prior to or simultaneously with the test sample to the element during the assay. Further details of useful antibodies for CK-MB determinations and carrier materials are provided, for example, in U.S. Patents 4,237,044 and 4,260,678.

The element of this invention comprises at least two layers which can be self-supporting, that is, having sufficient integral strength to remain intact during an assay. More generally, it comprises a nonporous support having thereon a first layer containing a photosensitive compound and a filter layer described herein. The layer containing the photosensitive compound generally comprises one or more binder materials in which the compound is distributed. Useful binder materials are known to one skilled in the art and include hardened or unhardened gelatin and other colloidal materials, polysaccharides and natural and synthetic polymers.

Alternatively, the photosensitive compound can be in the porous spreading layer (described above) of an element.

Similarly, the filter layer contains one or more filter dyes in one or more binder materials. The filter dyes used are chosen based upon their solubility in the binder, their extinction coefficient, the wavelengths of absorption and other parameters known to one skilled in the art. More particularly, the filter dyes are chosen to filter radiation which will adversely affect the photosensitive compound, but which will not filter radiation to which the compound is not sensitive. A mixture of such dyes can be used to absorb desired wavelengths. In a preferred embodiment, the filter dyes filter out radiation having a wavelength shorter than 500 nm. Examples of useful dyes are disperse textile dyes, ultraviolet light absorbers as known in the art. Examples of useful filter dyes include C.I. Disperse Red 137, C.I. Disperse Yellow 5, C.I. Disperse Orange 3 and 2,2'-dihydroxy-4,4'-dimethoxybenzophenone and others known in the art. All of these dyes are available commercially.

The amounts of filter dyes in the filter layer can be determined readily by a skilled worker in the art. The amount of each dye to be used depends upon the dye's extinction coefficient, its solubility in the binders used and the proportions needed to absorb the desired wavelengths. These amounts can be determined with routine experimentation.

The filter dyes can be used in a suitable binder material in which they are soluble and which can be suitably applied or otherwise incorporated into the element. Generally the binders are synthetic or natural polymeric or colloidal materials, for example, gelatin, agar, collagen, cellulose esters (such as cellulose acetate), polystyrene, polyurethane or polycarbonates. Cellulose acetate is preferred in the practice of this invention.

Alternatively, the filter layer can also serve as a nonporous support. The only critical aspect of the filter layer is that it is situated in relation to the photosensitive compound such that incident radiation used to detect the optical density change passes through the filter layer before reaching the photosensitive compound. The filter layer can be incorporated into the element simultaneously with preparation of the other layers. Alternatively, the other layers can be prepared first and the filter layer later applied to them in some manner, for example, coating or lamination.

More preferably, the element also includes a porous spreading layer as the outermost layer. Reagents and/or antibodies for isoenzyme determination can be incorporated into the porous spreading layer by imbibition, impregnation, coating

or another suitable technique. Generally, they are incorporated into a coating composition, whereas antibodies are incorporated by imbibition or wash coating into an already coated layer. Useful absorbent materials for making porous spreading layers are insoluble and maintain their structural integrity when exposed to water or biological fluids such as whole blood or serum. Useful elements can have spreading layers prepared from paper, porous particulate structures, porous polymeric films, cellulose, glass fibers, woven and nonwoven fibrous fabrics (synthetic and nonsynthetic). Useful materials and procedures for making such layers are well known in the art.

For example, the porous spreading layer can be prepared from any suitable fibrous or non-fibrous material or mixtures of either or both including those described in U. S. Patents 4,292,272, 3,992,158, 4,258,001 and 4,430,436 and Japanese Patent Publication 57(1982)-101760. The spreading layer should be isotropically porous, meaning that the porosity is the same in each direction in the layer as caused by interconnected spaces or pores between particles, fibers or polymeric strands.

The support can be any suitable dimensionally stable and nonporous, and preferably transparent (i.e. radiation transmissive) material which transmits electromagnetic radiation of a wavelength between 200 and 900 nm. A support of choice for a particular element should be compatible with the intended mode of detection (reflection, transmission or fluorescent spectroscopy). Useful supports can be made from paper, metal foils, polystyrene, polyesters, polycarbonates, cellulose esters and others known in the art.

The element of this invention can have a registration or reagent layer under the porous spreading layer. These layers can contain one or more reagents or enzymes needed for the assay, such as surfactants or buffers. They generally contain one or more hydrophilic binder materials (for example, treated or untreated gelatin and other colloidal materials, polysaccharides, vinyl pyrrolidone polymers or acrylamide polymers). Examples of other binder materials are known to one skilled in the art. Preferably, the layer contains gelatin which has been hardened with a standard hardener.

The elements can have one or more other layers, for example, additional spreading layers, radiation-blocking or filter layers, subbing layers, or barrier layers. The layers are generally in fluid contact with each other, meaning that fluids, reagents and reaction products can pass or be transported between superposed regions of adjacent layers by fluid.

A preferred embodiment of this invention is a multilayer element useful for determining CK-MB comprising a support having thereon, in order and in fluid contact on one side, a registration layer containing a photosensitive dye precursor (leuco dye) described herein and optionally other reagents, a reagent layer containing creatine phosphate, AMP, ADP and other desired reagents, optionally a subbing layer, and a porous spreading layer which optionally contains either a CK activator or at least one antibody for the M subunits of CK or both. The subbing layer can comprise one or more subbing materials known to one skilled in the art, for example, vinyl pyrrolidone polymers or acrylamide polymers.

When the preferred photosensitive dye precursor described above is used, the registration layer also contains α-glycerophosphate oxidase, and the reagent layer also contains glycerol and glycerol kinase.

On the other side of the support is a filter layer, described above, through which incident light passes prior to incidence upon the registration layer.

A variety of different elements, depending on the method of assay, can be prepared in accordance with the present invention. Elements can be configured in a variety of forms, including elongated tapes of any desired width, sheets, slides or chips.

The assay of this invention can be manual or automated. In general, in using the dry elements, analyte determination is made by taking the element from a supply roll, chip packet or other source and physically contacting it with a sample (for example, up to 200 µl) of the liquid to be tested so that the sample mixes with the reagents within the element. Such contact can be accomplished in any suitable manner, for example by dipping or immersing the element into the sample or, preferably, by spotting the element by hand or machine with a drop of the sample with a suitable dispensing means.

After sample application, the element is exposed to any conditioning, such as incubation, heating or the like, that may be desirable to quicken or otherwise facilitate obtaining any test result.

In the case of CK or an isoenzyme, CK or isoenzyme in the test sample catalyzes reaction of the ADP with the creatine phosphate substrate at a rate based on the amount of analyte present in the sample. The rate of optical density change (for example, dye formation) due to either reaction of

creatine phosphate or formation of the reaction product (for example, ATP) is quantifiable by passing the element through a zone in which suitable detection apparatus for reflection or transmission spectrophotometry is provided. Suitable detection apparatus and procedures are known in the art.

As used in the context of this disclosure and the claims, I.U. represents the International Unit for enzyme activity defined as one I.U. being the amount of enzyme activity required to catalyze the conversion of 1 micromole of substrate per minute under standard pH and temperature conditions for the enzyme.

Examples 1 and 2: Assay of Creatine Kinase-MB

In these examples, the amount of undesired rate of change in optical density was measured with an element of this invention. CK-MB was also determined in pooled human serum using the element. The assay of this invention was compared to an assay carried out with a known analytical element which is outside the scope of this invention.

The elements used in this comparison had the general format and components shown below. The Control element, however, did not have a filter layer. They were prepared like the element described in U.S. Patent 4,547,461, noted above except that it also contained anti-CK-MM antibodies in the spreading layer. The element of this invention comprised a filter layer having the composition shown below and coated on the backside of the support.

	Goat anti-human CK-MM	22,900 U/m^2**
	TiO_2	50 g/m^2
	Cellulose acetate	7 g/m^2
Spreading Layer	ESTANE 5715 resin	2.5 g/m^2
	N-acetyl-L-cysteine	0.4 g/m^2
	TRITON X-405 surfactant	1.7 g/m^2
	Ethylenebis(oxyethylene-nitrilo)tetraacetic acid	0.65 g/m^2
Subbing Layer	Poly(N-isopropylacrylamide)	0.4 g/m^2
	Gelatin (Hardened)	5.4 g/m^2
	Magnesium acetate	0.65 g/m^2
	TRITON X-200E surfactant	0.11 g/m^2
	Adenosine-5'-diphosphate (ADP)	0.08 g/m^2
	Glycerol kinase	4320 $I.U./m^2$
Reagent Layer	Adenosine-5'-monophosphate (AMP)	1 g/m^2
	Creatine phosphate	1.6 g/m^2
	P^1,P^5-di(adenosine-5')-pentaphosphate (DAPP)	0.05 g/m^2
	Glycerol	0.2 g/m^2
	2-[Bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol	1.5 g/m^2

	Gelatin (Hardened)	5.4 g/m^2
	2-[Bis(2-hydroxyethyl)-amino]-2-(hydroxymethyl)-1,3-propanediol	1.5 g/m^2
Registration Layer	ALKANOL XC surfactant	0.3 g/m^2
	Peroxidase	32,400 I.U./m^2
	2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl)imidazole	0.2g/m^2
	Ascorbic acid oxidase	10,800 I.U./m^2
	L-α-Glycerophosphate oxidase	3240 I.U./m^2
	Glycolic acid	0.3 g/m^2
	5,5-Dimethyl-1,3-cyclohexanedione	0.05 g/m^2
	TRITON X-200E surfactant	0.1 g/m^2
	2,4-Di-_n_-pentylphenol	2 g/m^2
	Poly(ethylene terephthalate) Support	

** The antisera level is given in Units (U) which are defined by the titer assay: (50% inhibition titer) (mℓ/0.093 m^2) = U/m^2.

The element of the present invention comprised a filter layer coated adjacent to the support on the side opposite the other element layers. This filter layer contained the following materials: cellulose acetate binder (10.7 g/m^2), UVINOL 490 ultraviolet filter dye (0.3 g/m^2), C.I. Disperse Orange 3 (EASTONE Orange 2R) filter dye (0.15 g/m^2), C.I. Disperse Yellow 5 (EASTONE Yellow 6GN) filter dye (0.35 g/m^2) and C.I. Disperse Orange 3 (EASTONE Red 2B-GLF) filter dye (0.15 g/m^2).

The elements (both Control and invention) were evaluated by applying a 10 µl sample of either distilled water or pooled human serum to the spreading layer, incubating at 37°C for up to 12 minutes, and measuring the change in reflection density resulting from dye formation with a spectrophotometer.

Reflection density readings were transformed into transmission density (D_T) readings using Clapper-Williams transforms [described in J. Opt. Soc. Am., 43, 595 (1953)]. A plot of transmission density versus time was made for each element. The results are shown in FIGS. 1 and 2. FIG 1 shows the background rate when the Control and invention elements were spotted with distilled water, whereas FIG 2 shows the background rate when the elements were spotted with pooled human serum. It can be seen that the Control element (absent a filter layer) exhibited a high background rate while the element of the present invention exhibited a significantly lower background rate. Both figures show the magnitude of the background rate change between the Control element and the element of the present invention.

Example 3: Alternative Analytical Element

Another element of the present invention was prepared similar to that shown in Examples 1 and 2 except that the filter layer was applied to the support adjacent to the registration layer. The element was evaluated as described in Example 1 and a calibration curve was generated using standard procedures by applying samples having predetermined amounts of CK-MB. This curve is shown in FIG 3.

Example 4: <u>Assay for Creatine Kinase-MB</u>

An assay for CK-MB was carried out using an element and the procedures described in Examples 1 and 2 above. The Control element of Examples 1 and 2 was similarly tested. The elements were spotted with two test solutions: pooled human serum containing CK-MB (about 300 I.U./l CK-MB), and a bovine serum albumin (BSA) solution containing about 2000 I.U./l CK-MM and about 40 I.U./l CK-MB.

From the reflection density results obtained, precision of both assays was calculated for both test samples. The precision results are shown in Table I below. It is evident that the assay of the present invention is more precise with both test samples than the assay using the Control element.

TABLE I

Element	Test Solution	Early Read % C.V.*	Late Read % C.V.*
Control	Human Serum	2.5	1.9
Control	BSA	14.6	16.3
Example 4	Human Serum	1.2	0.8
Example 4	BSA	6.1	5.5

*% C.V. = % coefficient of variation. The early read was made after about 4 minutes into the assay, and the late read was made after about 5.6 minutes into the assay.

Claims:

1. A self-supporting analytical element comprising an absorbent carrier material containing an interactive composition for a clinically significant enzyme analyte comprising a photosensitive compound,

characterized in that the element has a filter layer comprising at least one filter dye and situated in relation to the carrier material such that incident radiation for detecting the density change passes through the filter layer prior to incidence upon the photosensitive compound.

2. A multilayer analytical element containing an interactive composition comprising a photosensitive dye or dye precursor which is capable of providing an optical density change as a result of interaction with a clinically significant enzyme analyte, the element comprising a nonporous support having thereon one or more layers, one of which contains the photosensitive dye or dye precursor,

characterized in that the element further comprises a filter layer comprising at least one filter dye and situated in relation to the layer containing the photosensitive dye or dye precursor such that incident radiation for detecting the density change passes through the filter layer prior to incidence upon the layer containing the photosensitive dye or dye precursor.

3. The element as claimed in claim 2 wherein the support is transparent to incident radiation and the photosensitive dye or dye precursor is in a layer located on the opposite side of the support from the filter layer.

4. The element as claimed in claim 2 wherein the filter layer is located between the support and a layer containing the photosensitive dye or dye precursor.

5. The element as claimed in any of claims 2 to 4 wherein the interactive composition provides a detectable optical density change in response to creatine kinase.

6. The element as claimed in any of claims 2 to 5 wherein the photosensitive dye precursor is an imidazole leuco dye.

7. The element as claimed in any of claims 2 to 6 wherein the outermost layer on the support is a porous spreading layer.

8. The element as claimed in claim 7 wherein the interactive composition comprises adenosine-5'-diphosphate, glycerol, glycerol kinase and α-glycerophosphate oxidase and the element further comprises creatine kinase-M antibodies immobilized in the porous spreading layer.

9. A method for the determination of a clinically significant enzyme analyte comprising the steps of:

A. contacting a sample of a liquid suspected of containing a clinically significant enzyme analyte with an analytical element as claimed in any of claims 1 to 8, and

B. determining the optical density change resulting from the presence of the analyte.

10. The method as claimed in claim 9 wherein the detectable optical density change is determined at a wavelength of greater than 500 nm.

FIG. 1

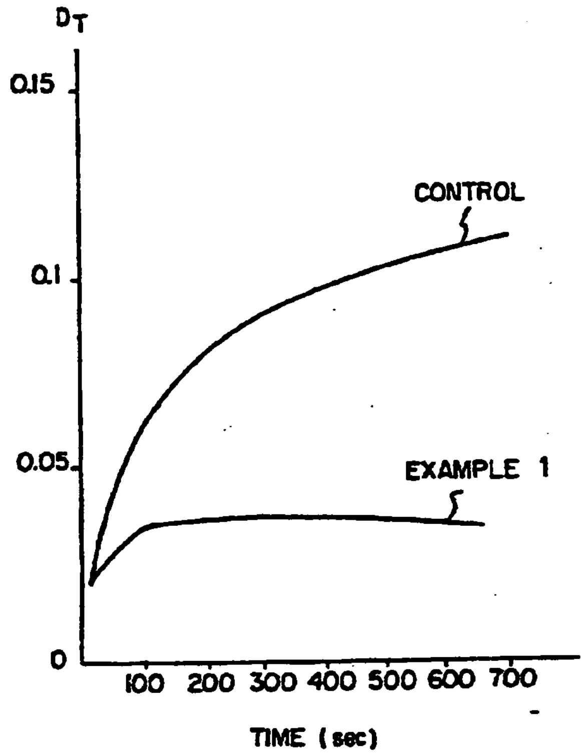
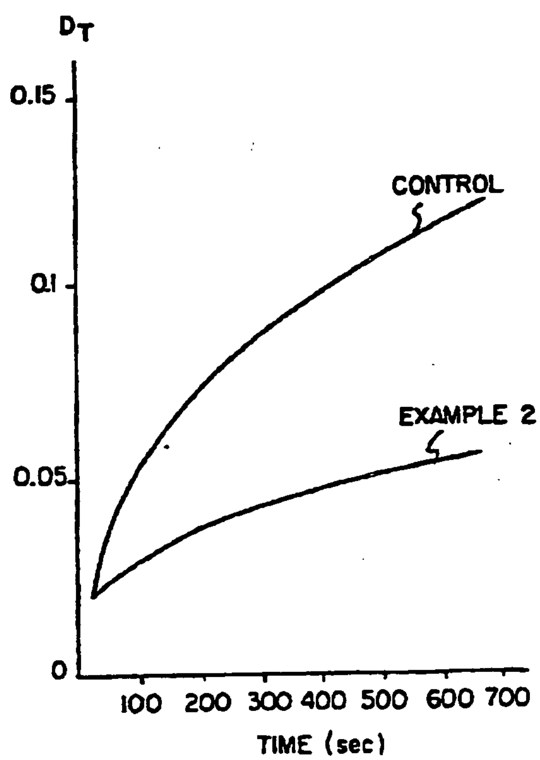

DT
0.15
0.1
0.05
0
CONTROL
EXAMPLE 1
100 200 300 400 500 600 700
TIME (sec)

FIG. 2


DT
0.15
0.1
0.05
0
CONTROL
EXAMPLE 2
100 200 300 400 500 600 700
TIME (sec)

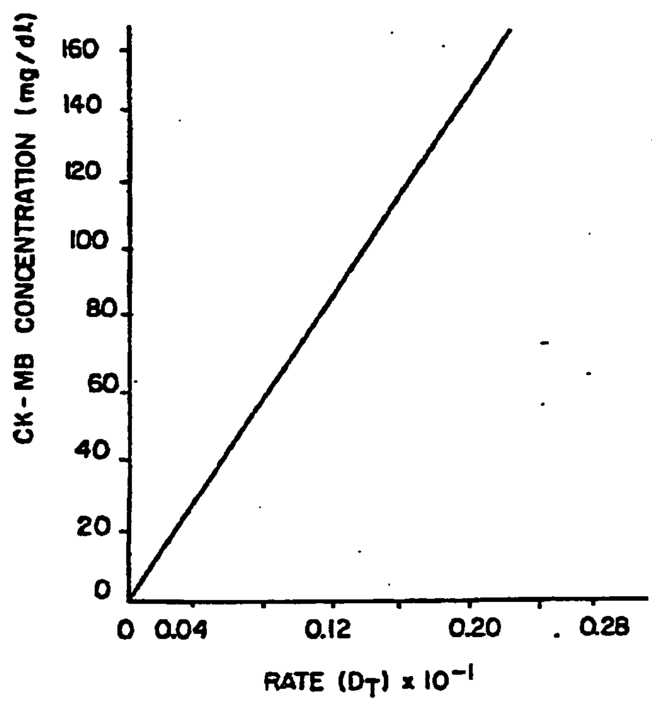

CK-MB CONCENTRATION (mg/dl)
160
140
120
100
80
60
40
20
0
0
0.04
0.12
0.20
0.28
RATE (D_T) x 10^{-1}

FIG. 3

European Patent Office

EUROPEAN SEARCH REPORT

0239222

Application number

EP 87 30 1331

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A,D	US-A-4 089 747 (B.J. BRUSCHI) * complete * ---	1	G 01 N 33/52 C 12 Q 1/00 C 12 Q 1/50
A	EP-A-0 116 307 (EASTMAN KODAK) * complete * & US - A - 4 547 461 (Cat. D) ---	1,8	
A	US-A-4 042 335 (P.L. CLEMENT) * column 1, example 1 * ---	1	
A	EP-A-0 092 688 (SAGAX) * claims 1-4 * ---	1	
A	EP-A-0 166 365 (FUJI PHOTO FILM CO.) * complete * ---	1	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
A	US-A-3 992 158 (E.P. PRZYBYLOWICZ) * claims * ---	1	G 01 N 33/00 C 12 Q 1/00
A	EP-A-0 033 707 (EASTMAN KODAK) * claims 1-4 * -----	1	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	21-05-1987	GREEN C.H.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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